A Plan for the Development of an Integrated Common Diagnostics System

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Program Framework

- Problem
- Requirement
- Solution

THE PROBLEM

(Medical Perspective)

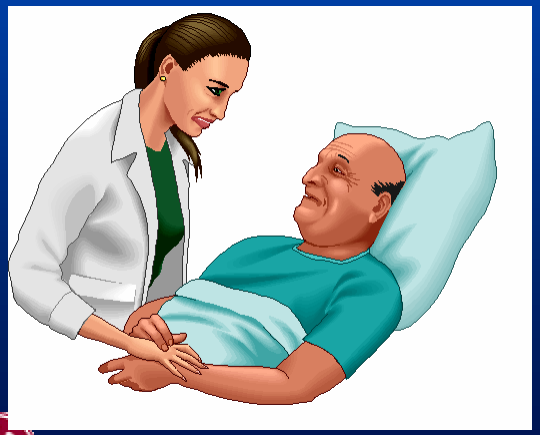
Diagnostics and Patient Care: Goals

- Reduce/prevent mortality
- Reduce/prevent morbidity
- Maintain unit readiness

Impact of Diagnostics on Patient Care

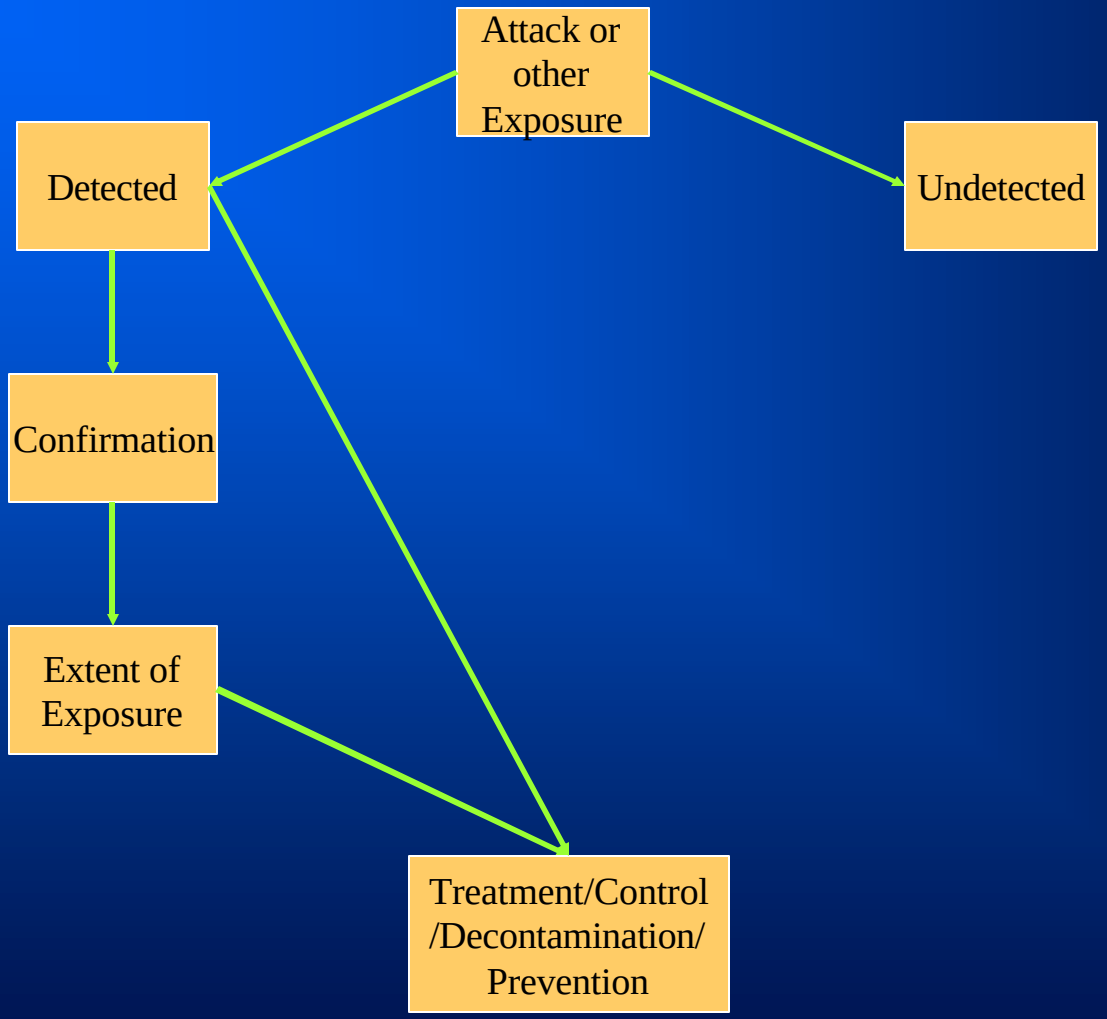
- **Immediate postexposure (up to 24 h)**
  - ✓ **very low concentration of agent**
  - ✓ **IMPACT**

- **Acutely ill**
  - ✓ **low concentration of agent**
  - ✓ **IMPACT**



- **Critically ill**
  - ✓ **High concentration**
  - ✓ IMPACT

Operational Scenario



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Diagnostic Selection Criteria

- Speed

Classical Methods for Identifying Biological Agents

- Culture Isolation (1 - 30 days)
- Animal inoculation (2 - 30 days)
- Antigen detection (4 - 18 hours)
- Antibody detection (2 hr - 10 days)

Shipping - 24 -72 hrs!

Diagnostic Selection Criteria

- Speed
- Sensitivity

Classical Methods for Identifying Biological Agents

- Culture Isolation
  - High
- Animal inoculation
  - High
- Antigen detection
  - Low to Moderate
- Antibody detection
  - High

Diagnostic Sensitivity Requirements

Agent	Infective Dose	Agent	Infective Dose
Anthrax	8,000 to 50,000 spores	Smallpox	10-100 organisms*
Brucellosis	10-100 organisms	VEE	10-100 organisms
Plague	100-500 organisms	Viral Hemorrhagic Fevers	1-10 organisms
Q-fever	1-10 organisms	Botulinum Toxins	~70 ng
Tularemia	10-50 organisms	Staph Enterotoxin B	~30 ng

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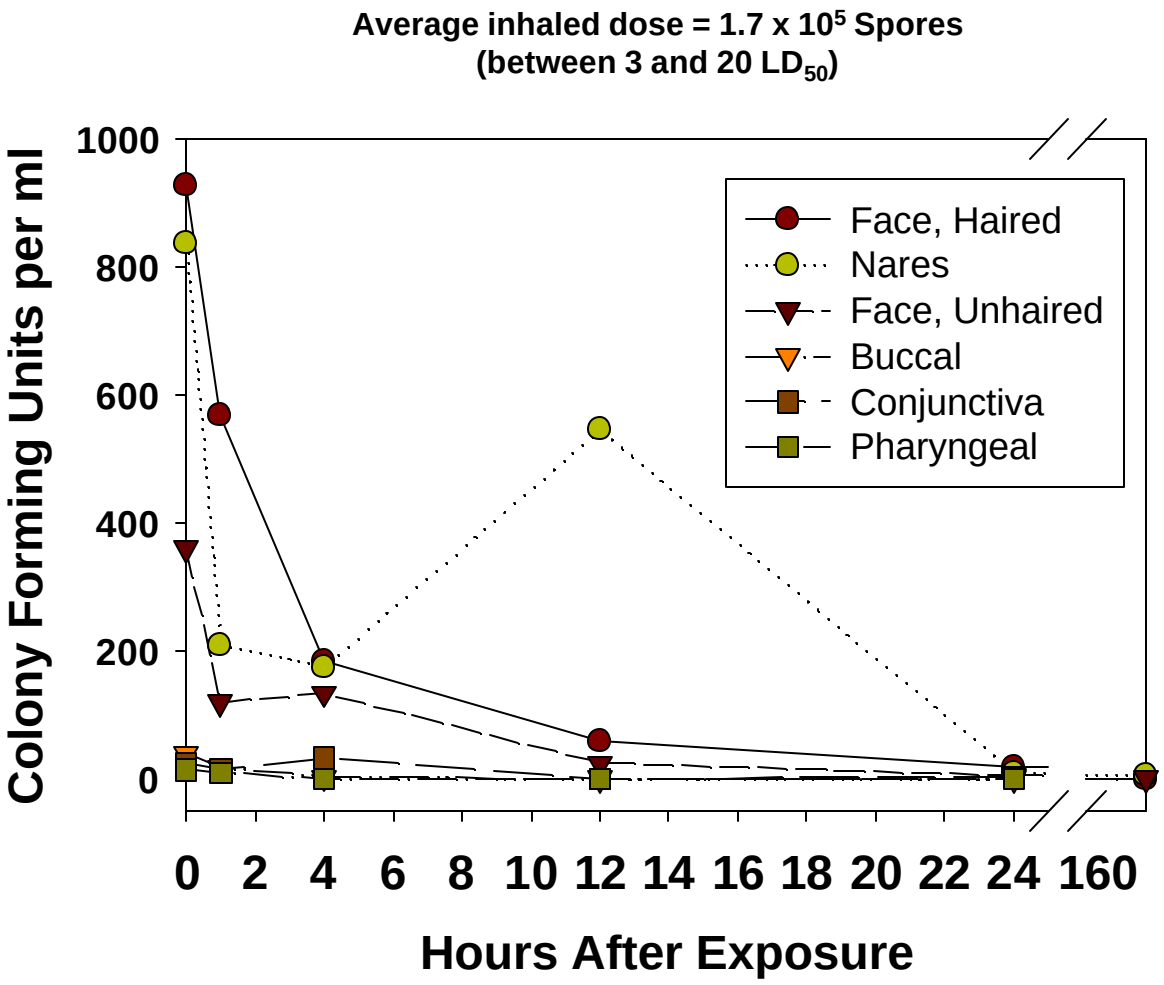
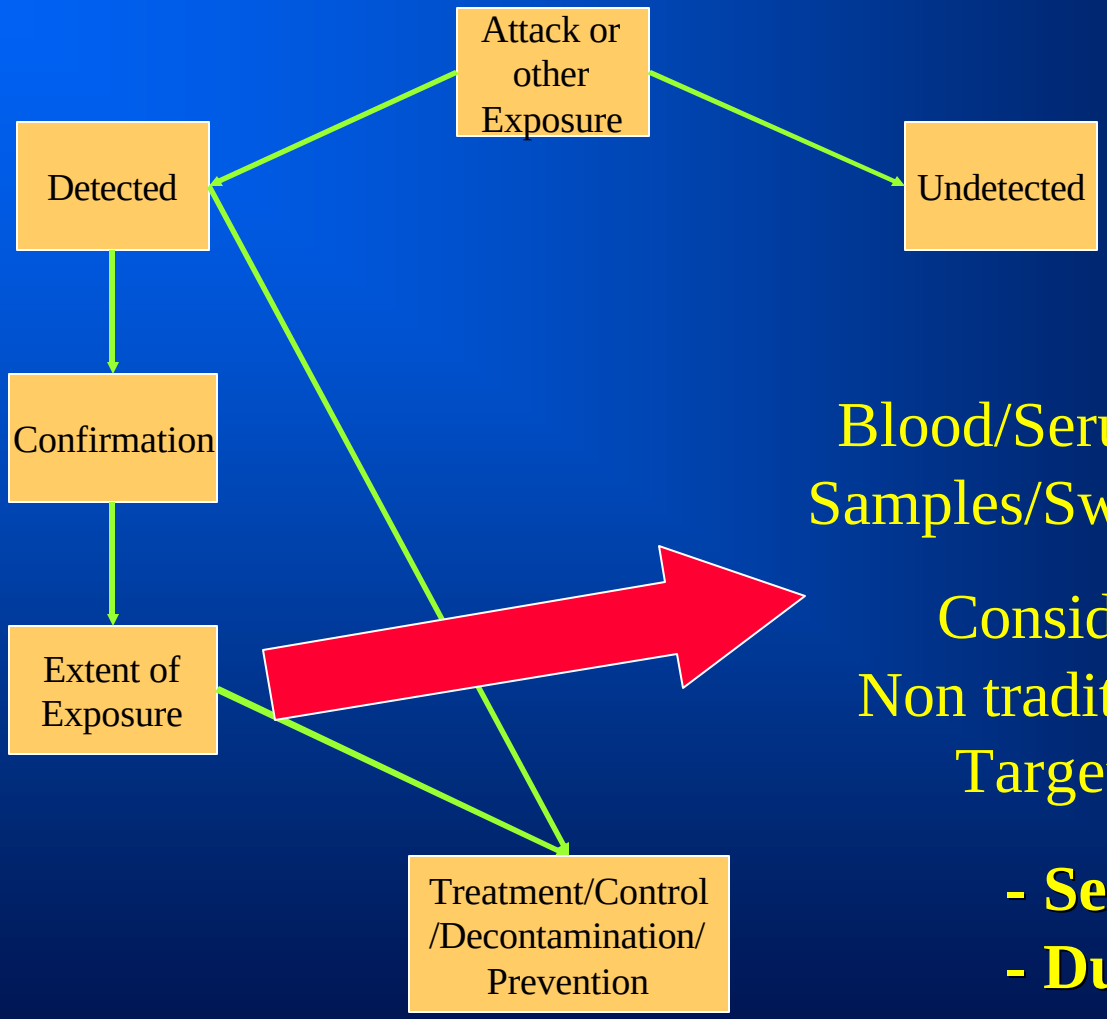
Anthrax in Swabs



Operational Scenario



Blood/Serum
Samples/Swabs

Consider
Non traditional
Targets

- Sensitivity?
- Duration?

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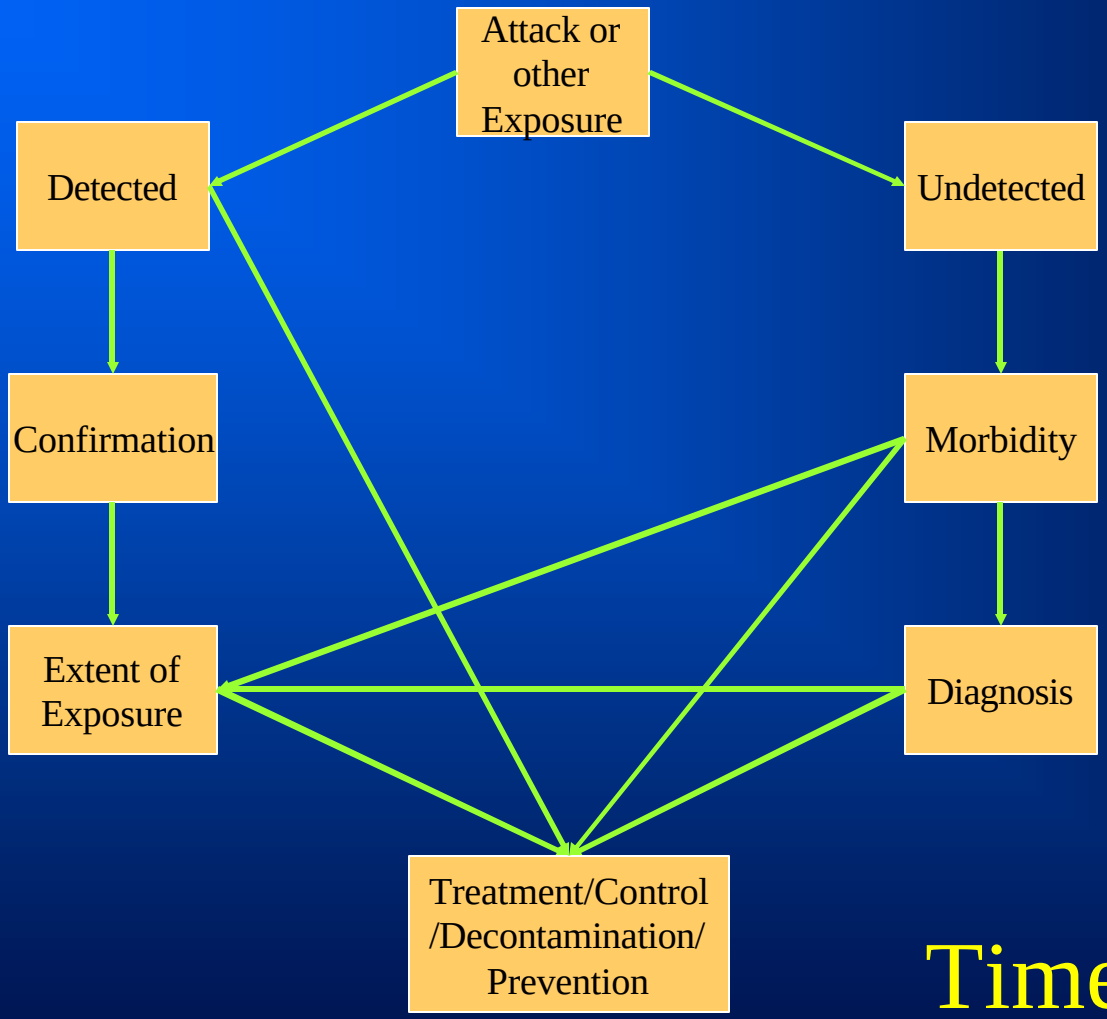
Operational Scenario



Time!

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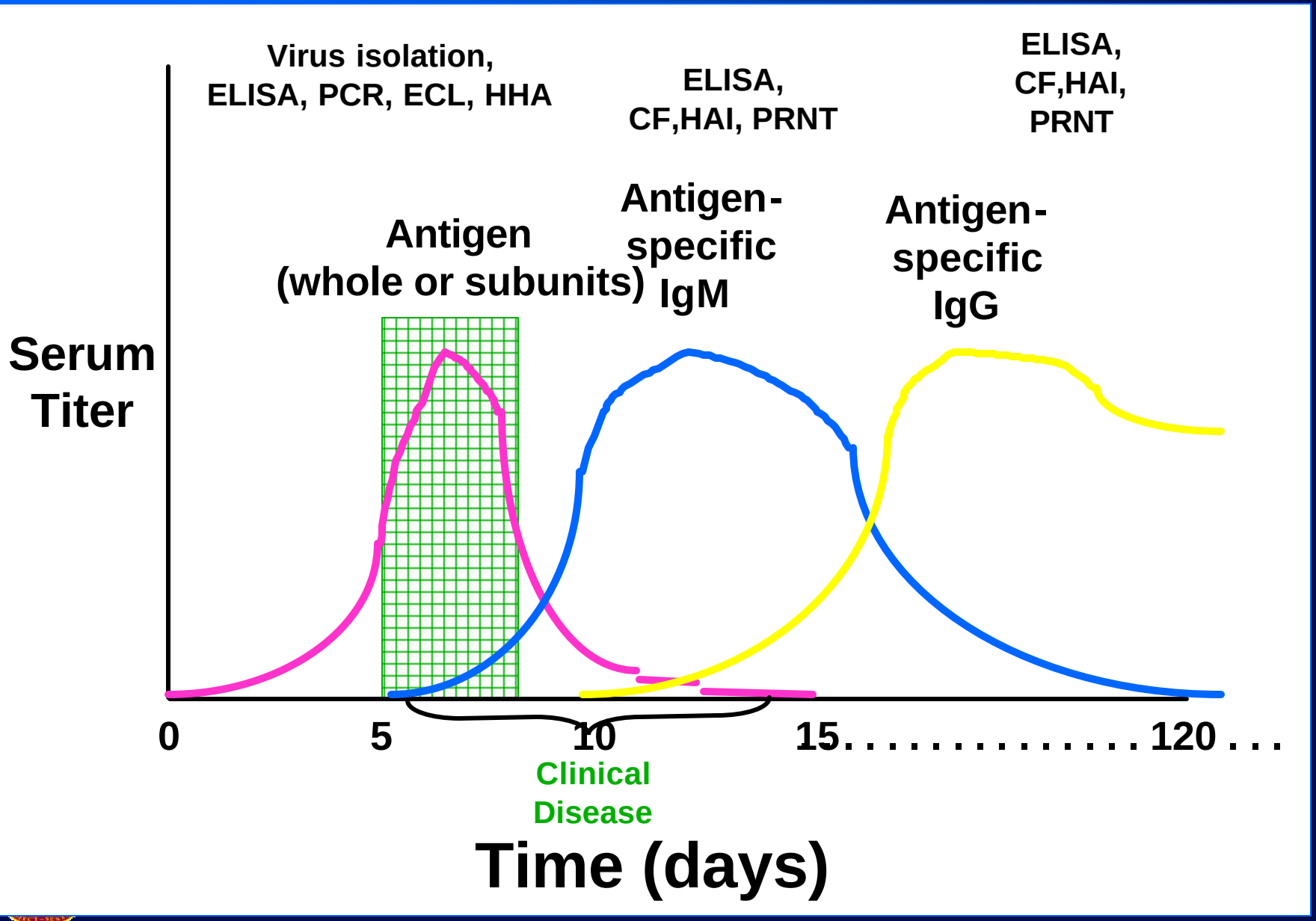
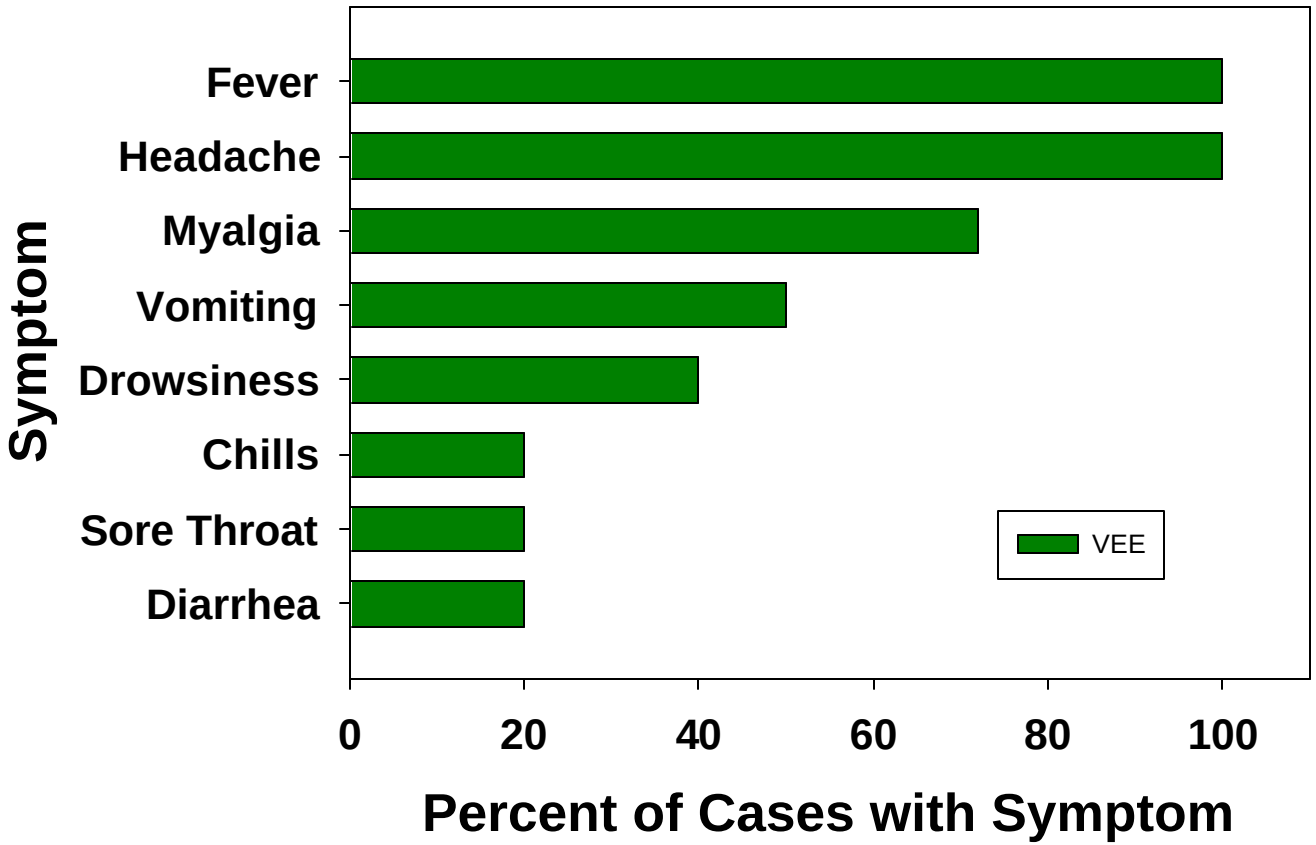
Diagnostic Markers

- Agent
- Antigen
- Antibody
- Nucleic Acid
- Cytokines/Chemokines
- Hormones
- Host Gene Expression

Diagnostics Time Curve -- VEE



Symptoms


Symptom
Fever
Headache
Myalgia
Vomiting
Drowsiness
Chills
Sore Throat
Diarrhea
VEE
0
20
40
60
80
100
Percent of Cases with Symptom

The Requirement

(DoD Perspective)

- Simultaneous identification >= 8 agents
- Identify biological agents at relevant conc.
- Sensitivity >= 0.98
- Specificity >= 0.98
- Approved or exempted by the FDA
- Upgradeable – equipment/assays
- Sample processing <= 20 min
- Total assay time <= 25 min
- Self-calibrating with failure alert

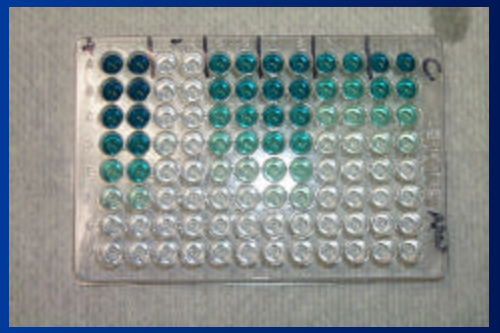
The Requirement

(DoD Perspective)

- Protect and preserve samples
- Software/Communications compatibility
- Set up <= 30 minutes
- Visual and audible positive identification signal
- Operable using all army power systems
- Battery operated >= 12 hours
- Back-up power capable
- Man portable
- Day/Night Operations.
- Onboard data storage.

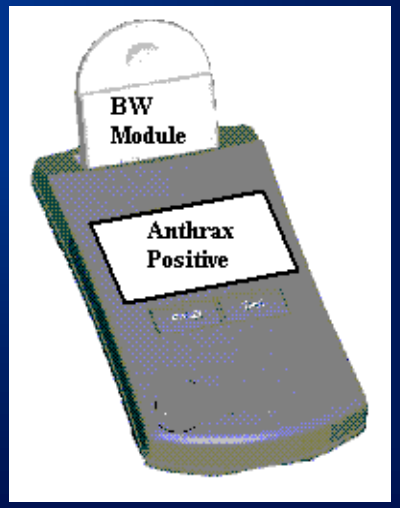
Comprehensive Integrated Diagnostic System

Automated Sample Processing



Immunodiagnostics

Nucleic Acid Detection



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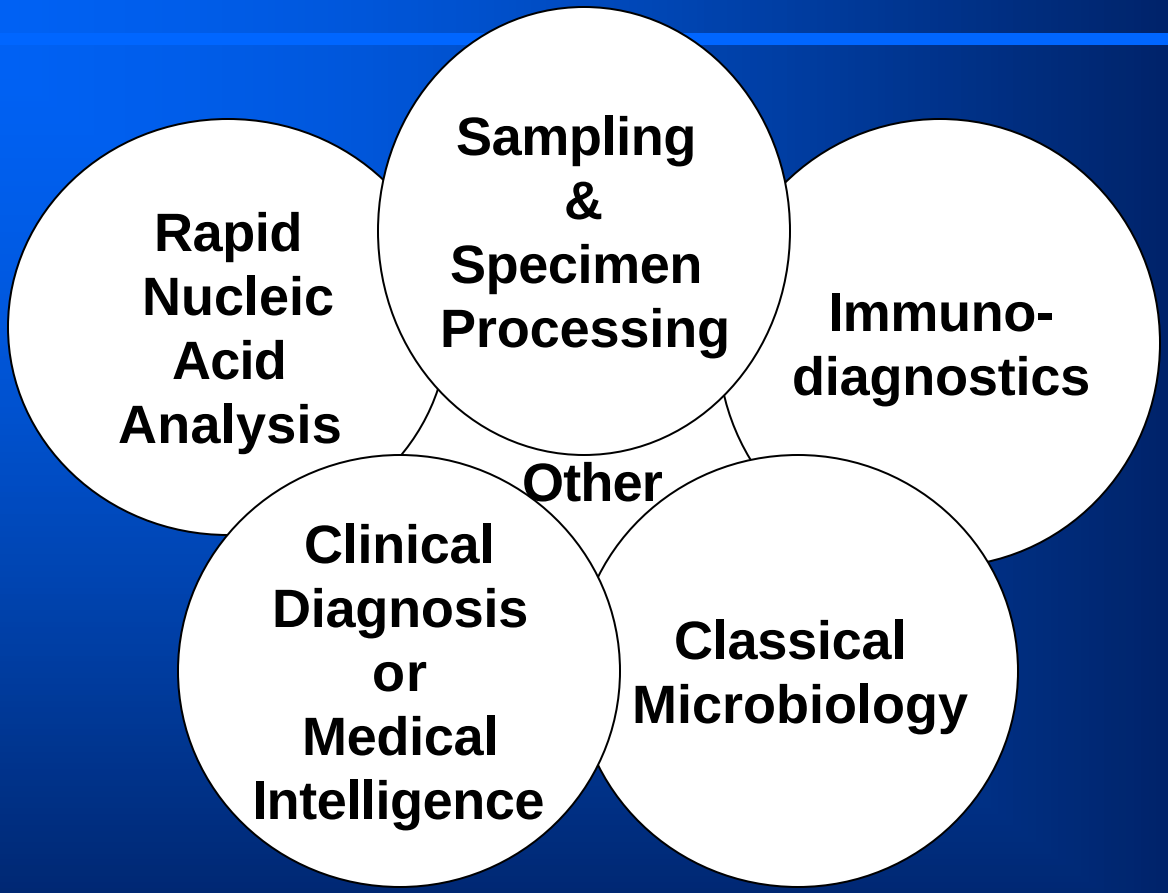
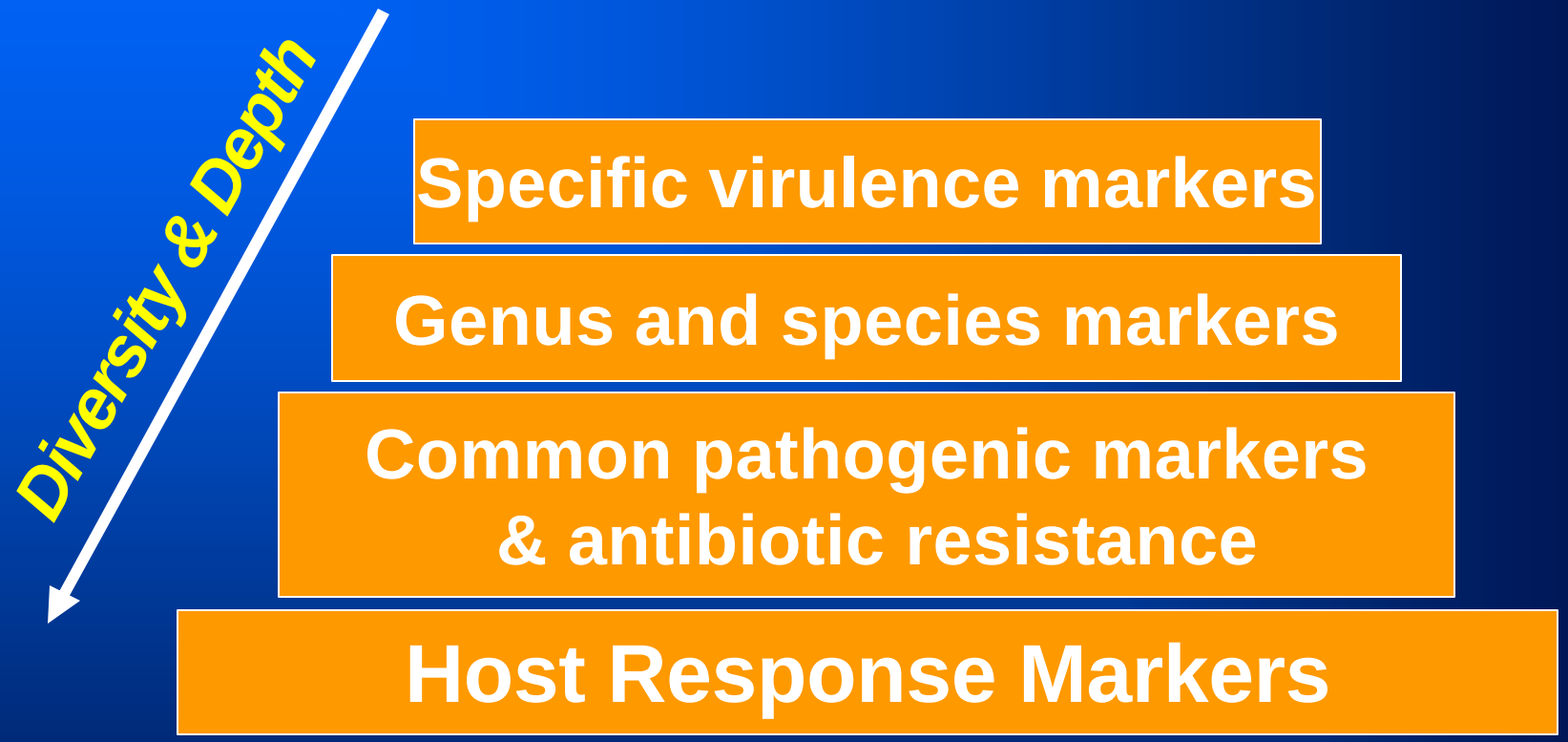
Integrated Identification and Diagnostic System



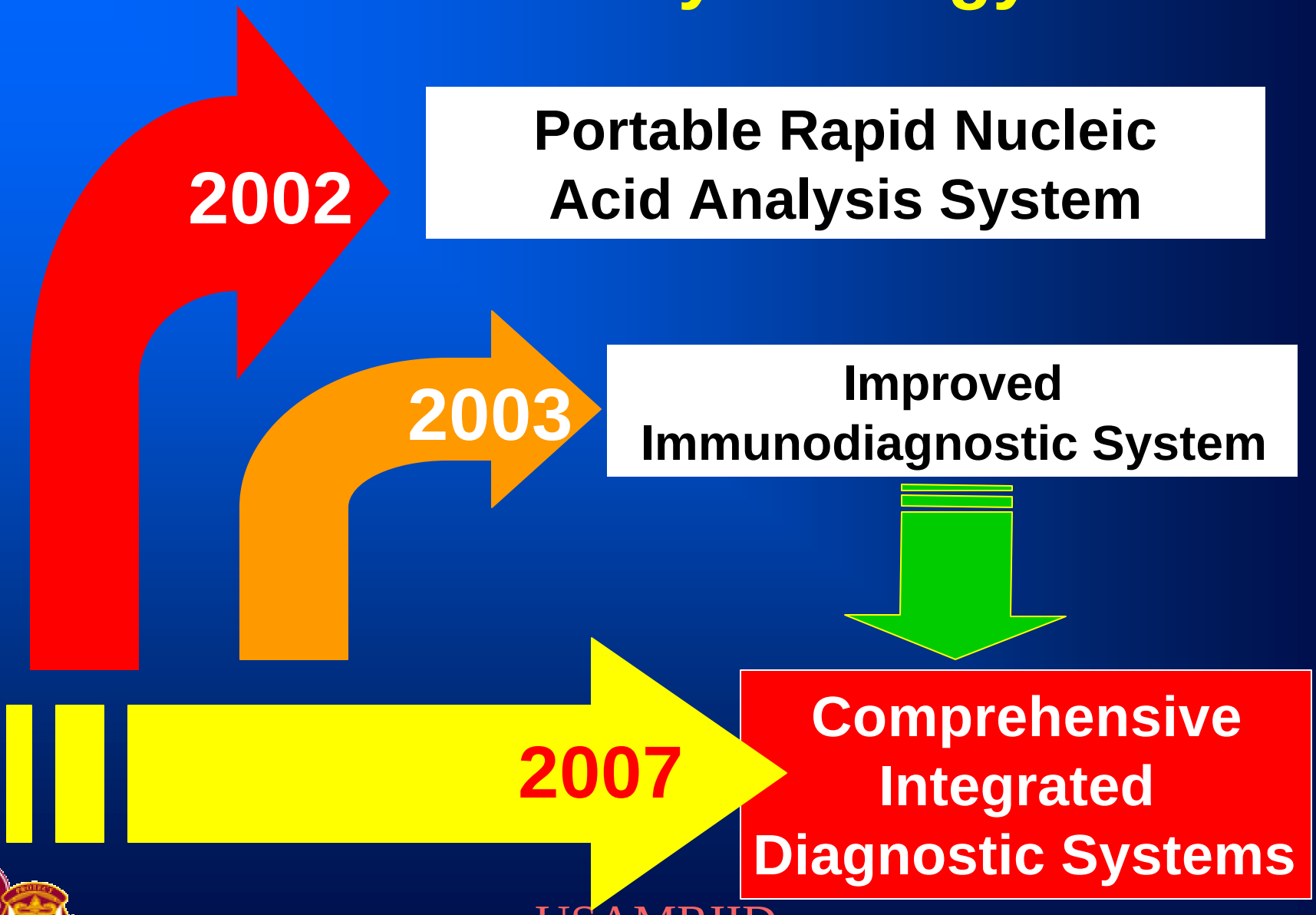
✓ ***Definitive biological agent diagnosis requires integrated identification technologies***

Biomarkers



✓ Avoid Technological Surprise

Evolutionary Strategy



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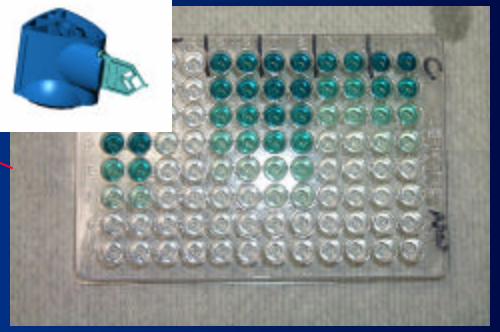
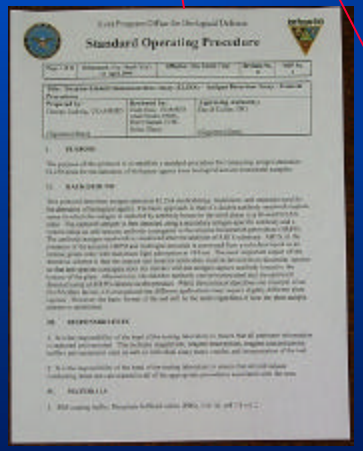
Medical Diagnostics

Platforms



Assays/Sample Processing

Reagents



Standardization

Validation

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Specimen Processing Challenge

Medical specimens

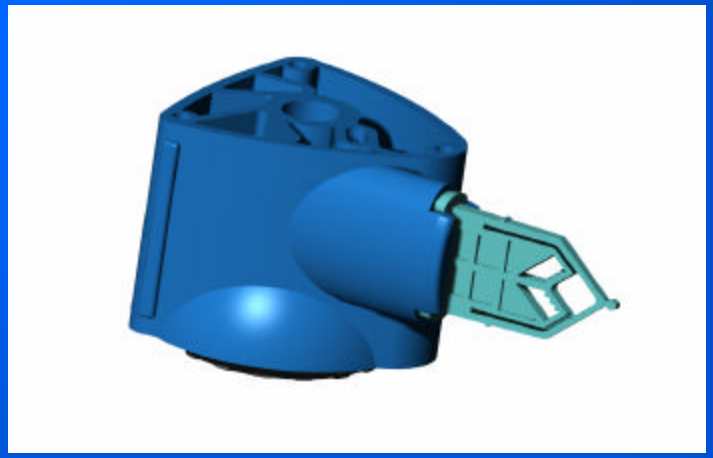
- swabs
- whole blood and serum
- urine
- feces
- sputum
- lesion exudate
- tissues

Environmental samples

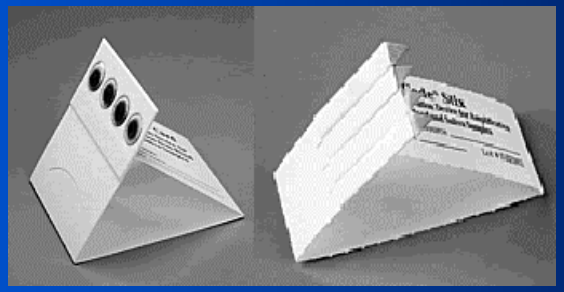
- high volume air sampler
- swabs
- water
- soil

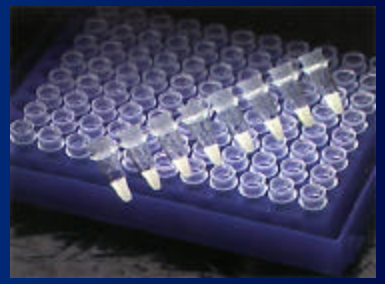
✓ Each matrix may require a unique processing protocol

Specimen Processing Options



Integrated Cartridge System



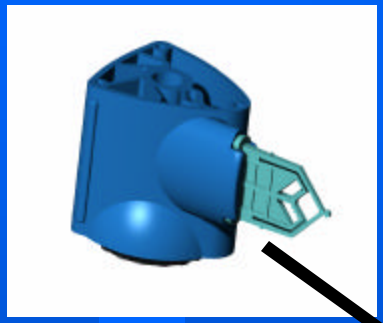


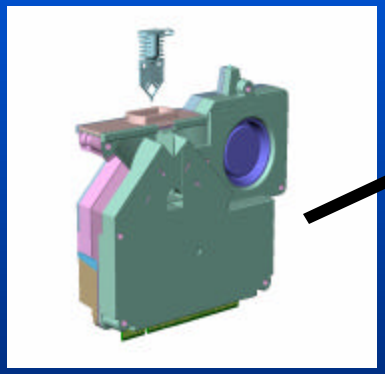
Tube and Paper Based Methods

Automated Systems

Manual Cartridge Systems

Gene Amplification and Detection Option





Four Cartridge
Integrated Nucleic Acid Analysis System

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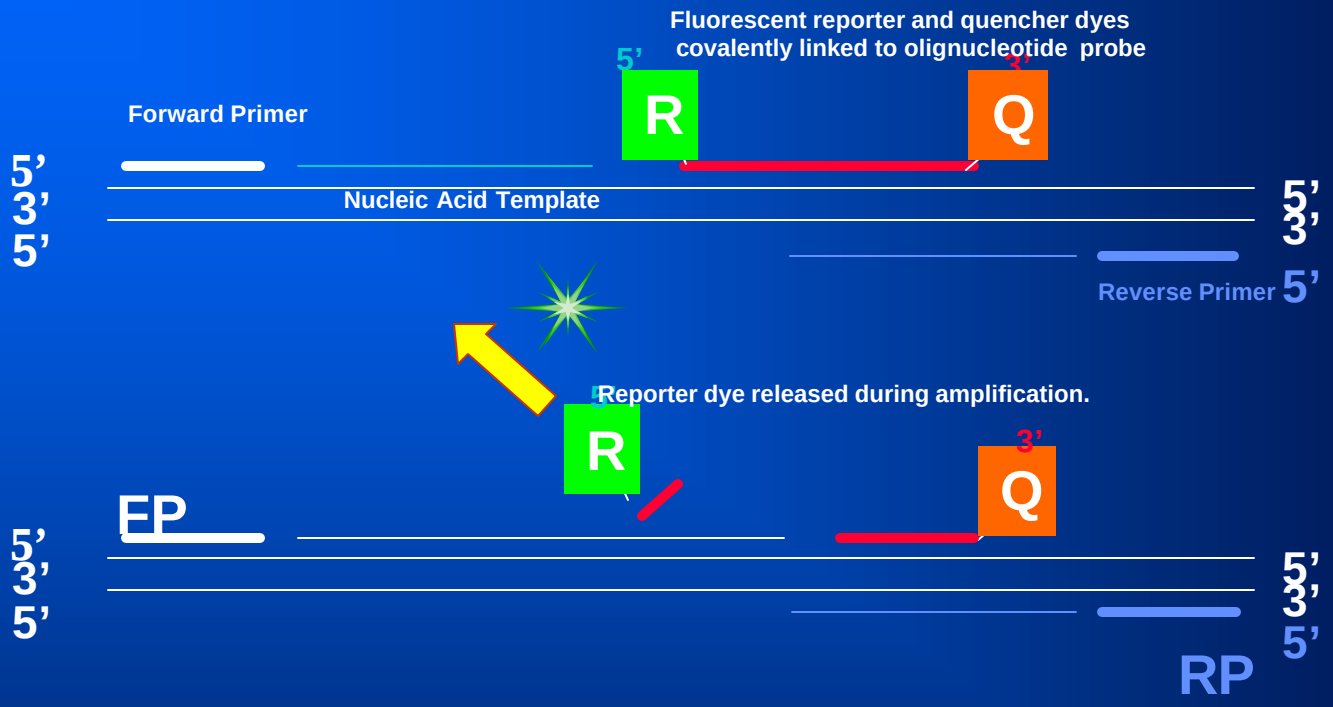
Common Gene Amplification Chemistry



- Common gene amplification chemistry
- Assays for over 26 biological agents
- COTS technology
- Over 50 assays developed

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Gene Amplification Reagents

- Pre-formulated
- Pre-dispensed
- Single-dose
- Ambient-temperature-stable
- Greater reproducibility
- Minimize errors and contamination

Tetracorp, Inc
Dried down reagents

•Amersham-Pharmacia
Ready-to-Go PCR
PCR-RT Beads
Custom BT Beads

- Idaho Technologies
Lyophilized in tubes

Leading Instrument Options

Smart Cycler™ XC

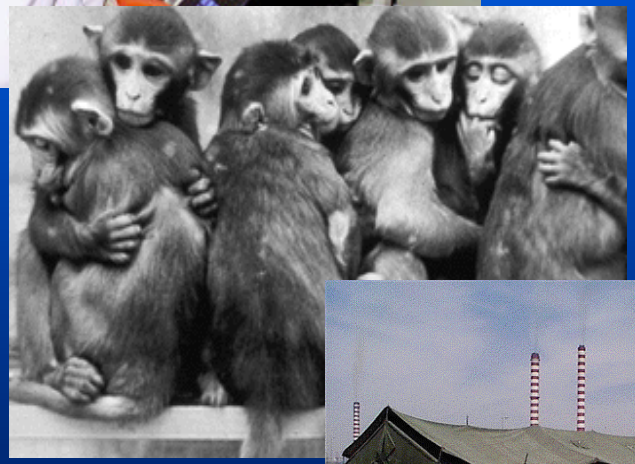
RAPID/LightCycler™

- Rugged and portable
- Rapid (25 to 40 mins after specimen processing)
- Sensitive
- Common fluorescent probe chemistry
- Different engineering and operation concepts

Device Comparisons

Characteristics	ABI 7700Ô	SmartCyclerÔ	R.A.P.I.D.S.
Optical Excitation	1 color	4 color	1 color
Optical Detection	4 color	4 color	2 color
Independent Thermocycling	no	yes	no
Reaction Volume	50 ul	100 ul	20 ul
Rapid Thermocycling	no	yes	yes
Low Power	no	yes	no
Average Analysis Time	2.5 to 3.0 h	20 to 40 min	20 to 40 min

Evaluation Trials



- Laboratory trials
- Animal models
- Field studies
- Hospital-based

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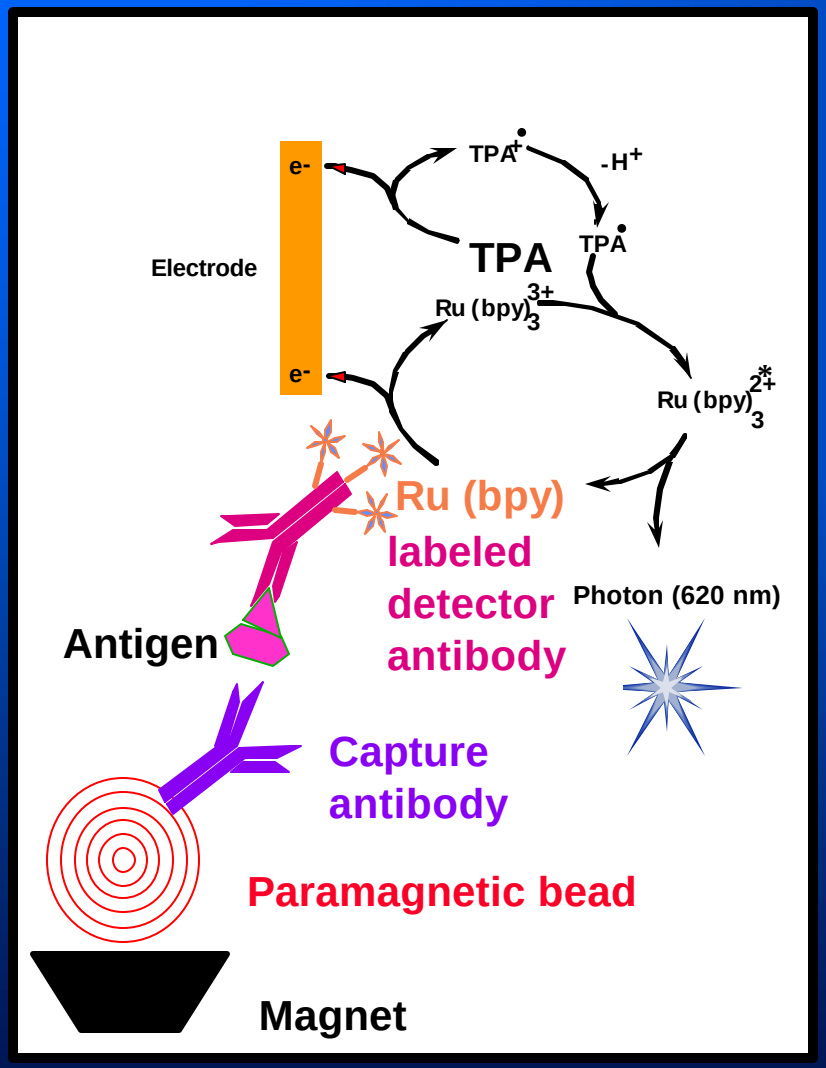
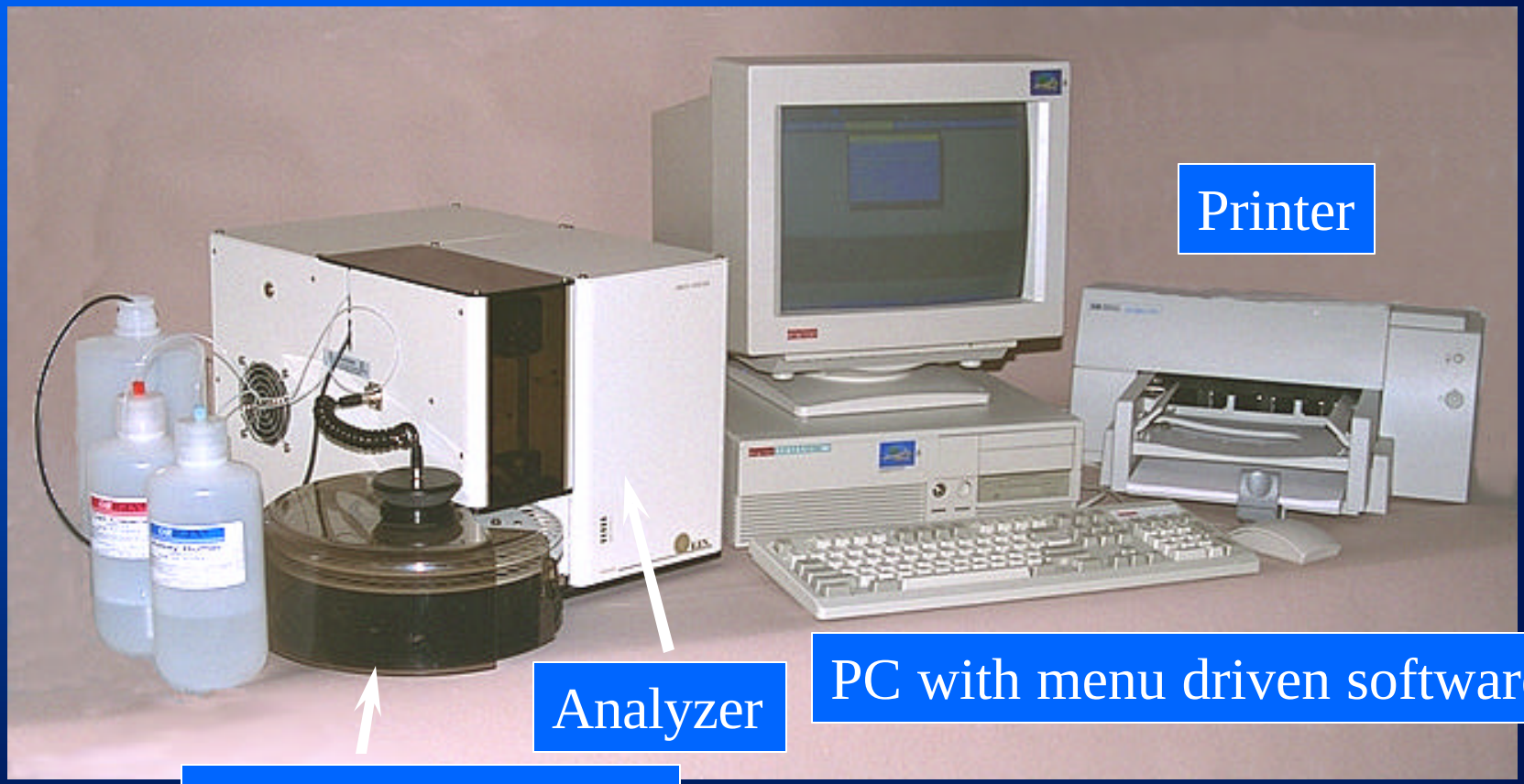
ECL Immunoassay



- High sensitivity
- Wide dynamic range
- 15 min assay
- Stable reagents

Electrochemiluminescence (ECL) Reaction
First Generation Device - ORIGEN®



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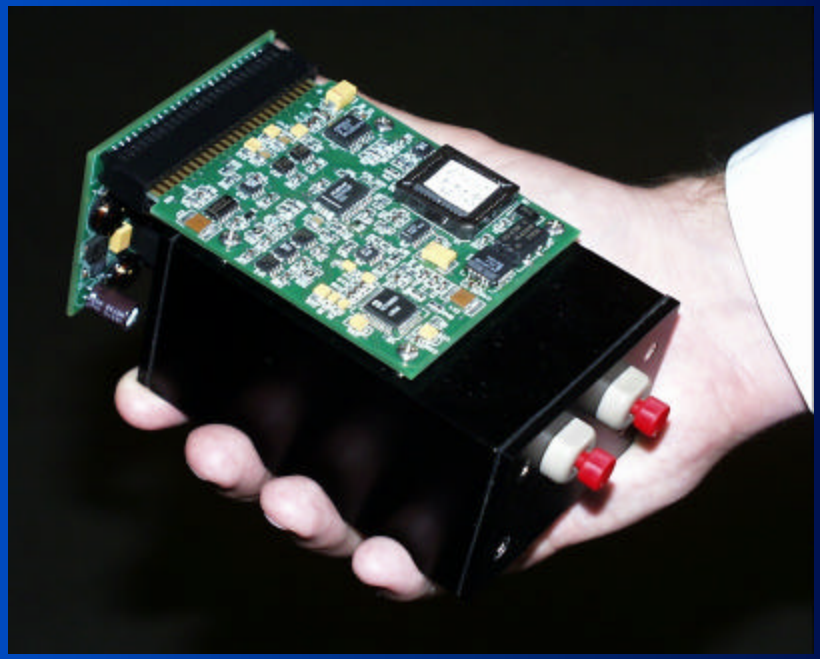
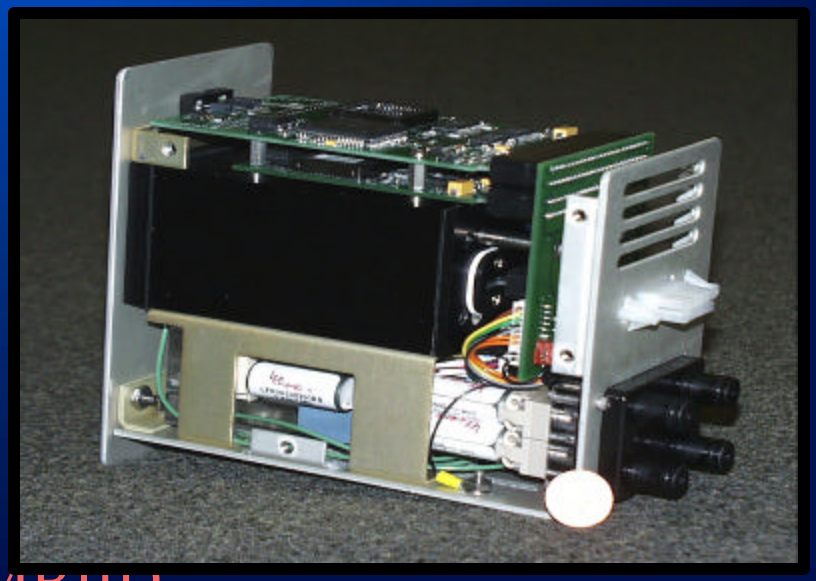
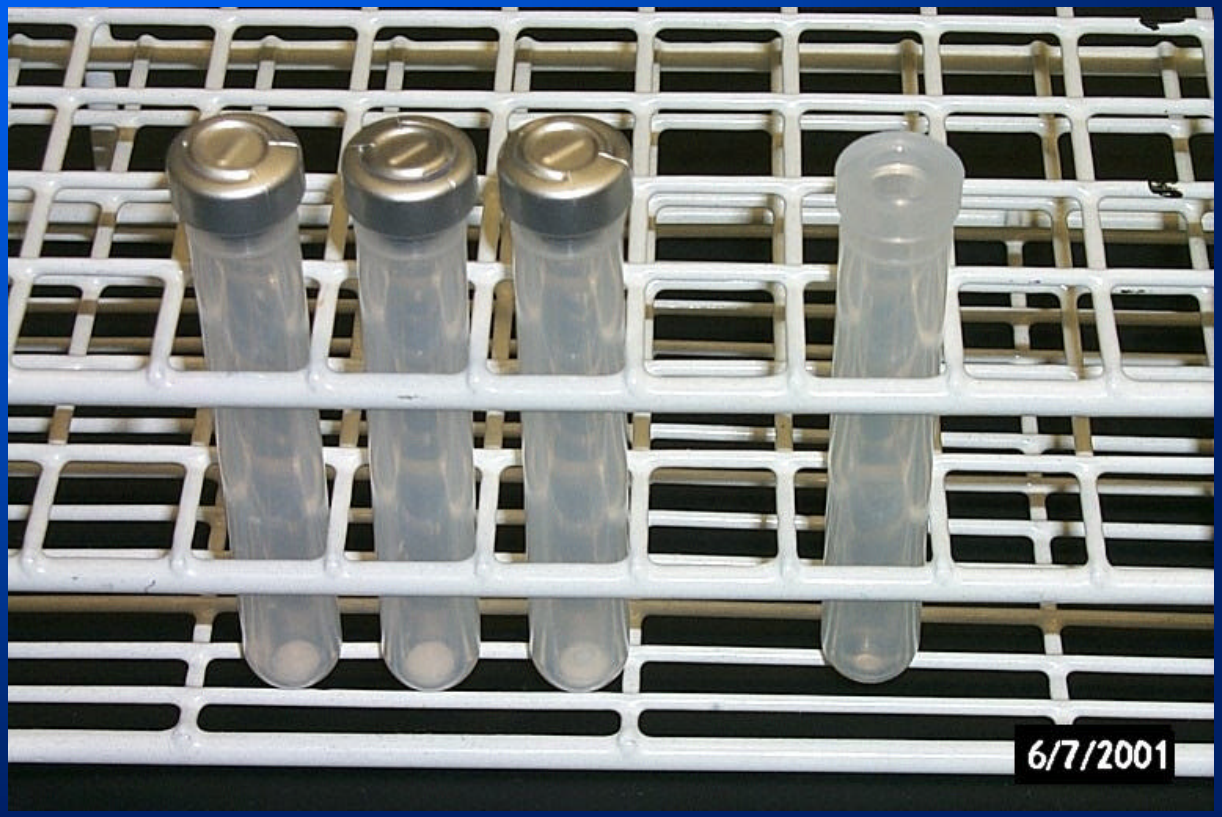
ECLM - Igen

- Modular design
- Conducive to portability
- Clinical device uses eight modules
  - 96 well plates
  - ease of maintenance





Single Tube ECL Assay



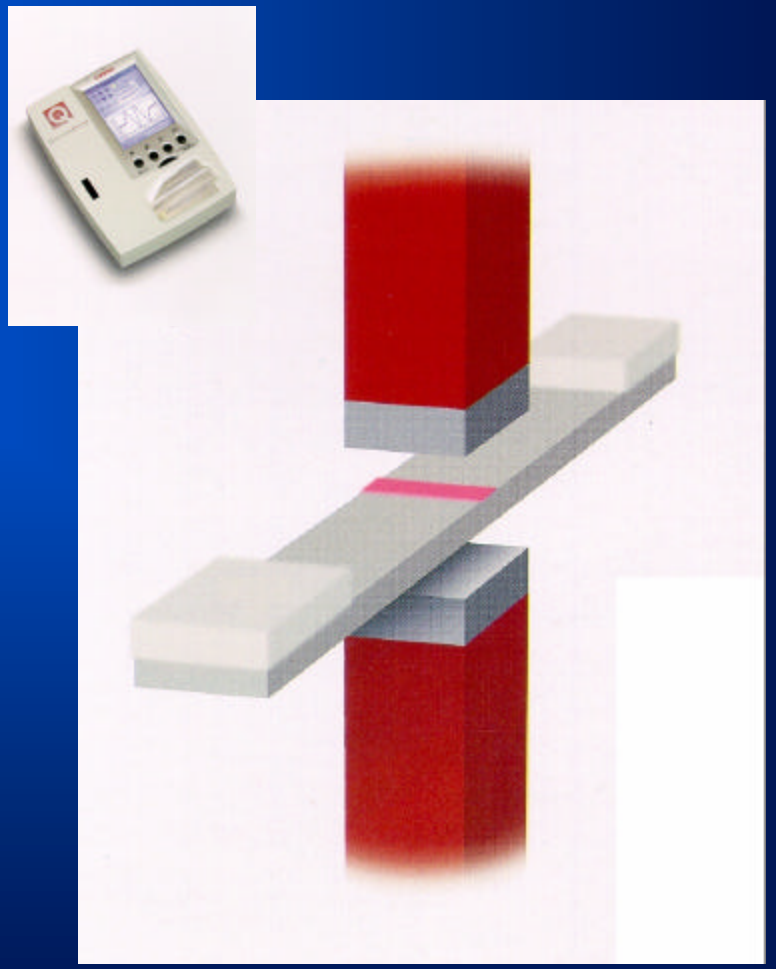
ECL Assays

- SEB Toxin
- Ricin Toxin
- Bot A Toxin
- Plague F1 Antigen
- Anthrax PA
- VEE Virus
- Orthopox

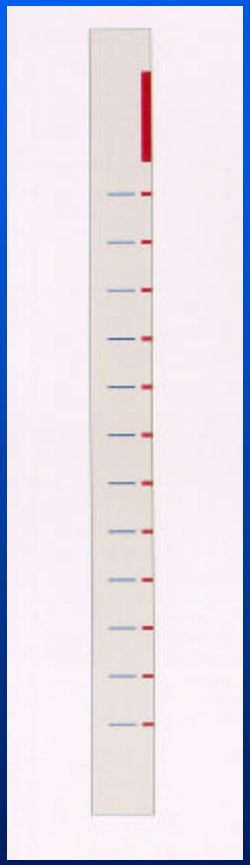
- Near Future
  - Fielding
    - TAML
    - Multi-center trials
  - Miniaturization and optimization of next generation device

Magnetic Assay Reader

- Measures the amount of magnetic material in the analytical region
- Oscillating magnetic field used to excite paramagnetic particles
- Detector measures the local magnetic field



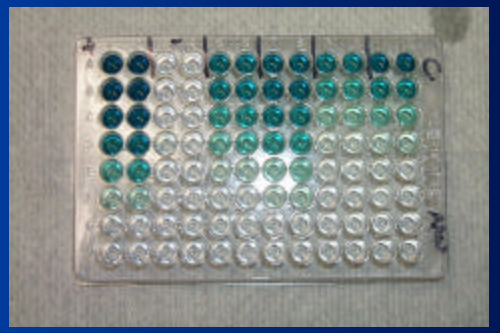
Hand Held Assay Strip



- Simple
- Multiple analyte testing possible
- 10-15 min test

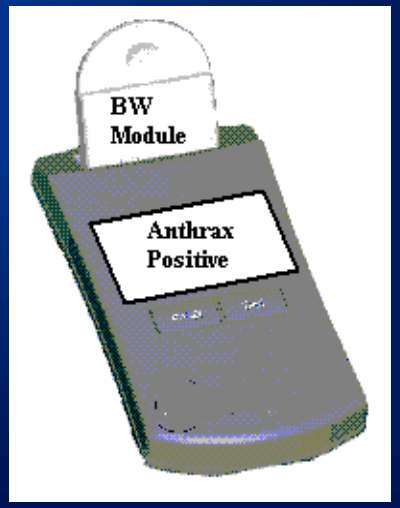
Comprehensive Integrated Diagnostic System

Automated Sample Processing



Immunodiagnostics

Nucleic Acid Detection



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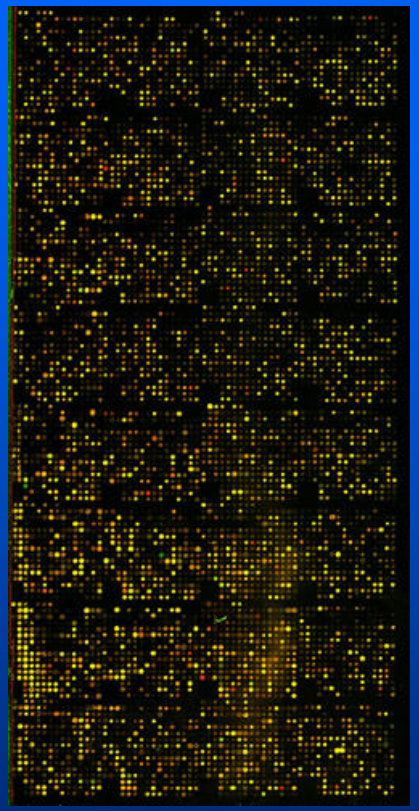
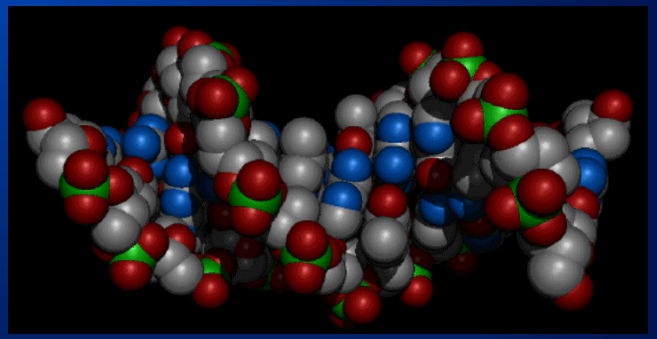
Far Term Development



Chip Technology

Mass Spectrometry



Advanced Nucleic Acid Sequencing

Summary

- Emerging technologies will eventually allow for the development of light weight hand-held devices
- Near term development is focused upon new methods of amplification of gene products and detection of immune complexes
- Far term development may take advantage of microchip, mass spectrometry, and other technologies with broad applications